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LEAD IN THE WATER SUPPLY ALTERS SWIMMING-MAZE BEHAVIOR IN ADULT MICE[1]

K. MCLEAN, G. H. PARKER, M. A. PERSINGER[2]

Laurentian University

Summary.—After about two weeks of exposure to either 20 ppm or approximately 2000 ppm of lead in the drinking water or tap water only and under an *ad libitum* or restricted food regime, albino male and female mice ($N = 48$) were tested for three consecutive days (3 blocks of 3 trials per day) in a swimming maze. Body weights were not altered by lead treatments significantly. The mice treated with the lead displayed longer escape latencies and more errors than the controls on tap water. Statistically significant interactions of lead treatment by test day by test block were also apparent.

Many behavioral studies concerned with potential lead toxicity in adult animals have involved intraperitoneal injections (Bullock, *et al.*, 1966; Brown, Dragann, & Vogel, 1971; Schmidt & Czech, 1977). Lead compounds have been administered through the food (Quarterman, Morrison, & Humphries, 1976) or the water supply (Ogilvie, 1977). All of these studies involved relatively large concentrations of lead or reported the potentially confounding effects of body-weight loss by the treated animals.

Most of these studies involved single test measures or simple statistical designs. Although these procedures may allow discrimination of large effects, they are less likely to detect subtle interactions between lead treatments and behavioral processes (such as learning) that require repeated measures to become evident. The present experiment was designed to determine the possible interactions between low-level lead (20 or $\sim$ 2000 ppm) treatments (delivered through the water supply) and swimming behavior by employing multivariate, repeated-measures designs.

METHOD

Subjects

Subjects were 24 female and 24 male, 60-day-old, Swiss albino mice (*Mus musculus L.*) obtained from Canadian Breeding Farms (Québec).

Procedure

Pairs of mice of the same sex were maintained throughout the experiment in 28.5-cm $\times$ 17.5-cm $\times$ 12.5-cm plastic cages containing corncob bedding. After 2 wk. of habituation to colony conditions (light:dark ratio 12:12, with light onset 0800 hr.; ambient temperature 21 $\pm$ 1°C) pairs of

[1]Supported in part by Canadian NSERC Grant No. A0445.
[2]Send reprint requests to Dr. M. A. Persinger, Neuroscience Laboratory, Department of Psychology, Laurentian University, Sudbury, Ontario, Canada P3E 2C6.

mice were assigned to one of three lead treatments (high, low and control dosages) and to one of two food conditions (*ad libitum* versus restricted feed).

Animals in the low-dosage group received 0.1 mM (39 μg/ml of lead acetate; 20 ppm Pb) in their drinking water for the remainder of the experiment. Animals in the high-dosage group received 0.01 M (3.8 mg/ml) in their drinking water. Since there was some precipitation of insoluable lead salts with the tap water vehicle, the concentration was less than 2000 ppm of Pb. The controls received the tap water only ($<<$ 1 ppm). Half the number of animals in each lead treatment were allowed *ad libitum* food options while the other half were given access to food for 1 hr. every 12 hr. (Pilot work indicated that the 23-hr. food deprivation resulted in death in some mice within six days of the schedule.) Body weights were taken once a week.

Ten days after the beginning of the treatments subjects were given three successive days (between 1200 and 1500 hr.) of testing in a T-shaped swimming maze (75 cm $\times$ 35 cm). The maze was constructed of glass and was immersed in 9 cm of water within a temperature controlled (30°C) water bath. The glass escape platform was submerged 1 cm beneath the water surface at the far end of the left arm of the maze.

Subjects were given nine trials per day with an intertrial interval of 30 sec. At the beginning of each trial the subject began swimming in the start area. The time required for the animal to swim the maze and climb on the escape platform after the gate had been opened was recorded to the nearest 0.1 sec. with a stopwatch. The subject remained on the platform for 25 sec.

Numbers of errors, defined as (1) any turn into the wrong arm of the maze and (2) a return or turn-around in the long arm of the maze, were also recorded. If the subject did not reach the platform within 10 min., it was removed and placed on the escape platform for 30 sec.; it was then returned to the start box for the next trial. Body weights were taken at the beginning of each session. The experiment was completed (during July-August, 1980) in four replicates, separated by 1 wk. with 12 subjects (two from each treatment and food condition) per replicate. Subjects in a replicate were of the same sex. The gender order in the replicates was M, F, M, F. All subjects were tested under non-blind conditions. One male in the low-lead, food-restricted group died during the experiment.

For analyses, the daily trials were collapsed into three blocks; the average escape latency and total errors for Trials 1-3, 4-6, and 7-9 were used. Since extreme values in some of the latency and error measurements resulted in skewness values much greater than 1.00, square root values were taken for all measures. In addition, a separate data set for latencies only was constructed by

truncating all values beyond 120 sec. All numerical properties of the measurements, and data transformations were completed by SPSS software using a DEC2020 System computer.

Four-way (three lead treatments by two food schedules) and five-way (three lead treatments by two food schedules by two sexes) analyses of variance with two factors repeated (three days and three blocks/day) were completed for the absolute latencies, square roots of the latencies, truncated latencies, absolute errors and square roots of the errors using BMD software (BMD P2V). Covariance for body weight was also completed. In addition, extra analyses with sexes collapsed and replication number as the third treatment were completed. Because of the discrepancies in standard deviations within the lead treatments (even despite data transformation), non-parametric Kruskal-Wallis tests were applied to data for the main dose effects. Four-way analyses of variance with one factor (day) repeated were completed for body weights during the treatment and test periods.

RESULTS

Means and standard deviations for the truncated and square roots of the average latencies (in sec.) and for the actual and square roots of the total errors per block are shown in Table 1 for the three main effects (lead, food

TABLE 1

MEANS AND STANDARD DEVIATIONS FOR MEAN TRUNCATED ESCAPE LATENCIES IN SECONDS PER BLOCK AND TOTAL ERRORS PER BLOCK FOR EXPERIMENT I

	Lead Treatment		
	Control ($n = 16$)	20 ppm Pb ($n = 15$)	2000 ppm Pb ($n = 16$)
Latency	7.1± 3.7 (2.5±0.5)	16.0±14.5 (3.6±1.7)	13.1±11.1 (3.2±1.1)
Errors	5.9± 2.0 (2.4±0.6)	6.7± 2.2 (2.5±0.5)	7.0± 2.2 (2.6±0.6)
		Food Condititon	
		Ad Libitum ($n = 24$)	Restricted ($n = 23$)
Latency		11.1± 6.9 (3.0±0.8)	12.8±14.2 (3.2±1.6)
Errors		6.7± 1.7 (2.6±0.5)	6.3± 2.5 (2.4±0.6)
		Gender	
		Male ($n = 23$)	Female ($n = 24$)
Latency		13.5±11.5 (3.2±1.2)	10.6±10.7 (3.0±1.3)
Errors		6.7± 2.5 (2.5±0.7)	6.4± 1.7 (2.5±0.4)
		Days	
	1 ($n = 47$)	2 ($n = 47$)	3 ($n = 47$)
Latency	10.8± 8.6 (3.1±1.1)	11.1±10.9 (3.1±1.3)	14.2±19.5 (3.3±1.9)
Errors	6.6± 2.5 (2.5±0.6)	6.3± 2.2 (2.5±0.6)	6.6± 2.6 (2.5±0.8)
		Blocks	
	1 ($n = 47$)	2 ($n = 47$)	3 ($n = 47$)
Latency	12.8±11.9 (3.2±1.3)	11.1±11.9 (3.0±1.3)	12.1±16.4 (3.1±1.8)
Errors	6.0± 2.5 (2.4±0.6)	6.9± 2.8 (2.5±0.7)	6.7± 2.7 (2.5±0.7)

Note.—Means and *SD*s for the square roots of the absolute latencies and total errors are shown in parentheses.

and sex) and the two (days and blocks) repeated measures. Since there were no significant effects of gender or interactions for either the latencies or the errors, only the results of the four-way analyses are reported. (The second F values are for the square root analyses.)

Small but statistically significant ($p \leq .05$) lead differences were noted ($Fs = 3.03, 3.23$; $df = 2/41$) for both latency measures. *A posteriori* analyses using Duncan's multiple-range test indicated the difference was primarily between the control group versus the low- and high-lead concentration groups but not between the latter two groups. Since homogeneity of variance tests indicated significantly ($Fs > 10.00$, $p < .01$) larger variance of scores within the lead groups relative to the control group, nonparametric tests were completed. Kruskal-Wallis tests also demonstrated statistically significant ($p < .05$) differences between the three lead treatments for both the truncated and square root of the latencies (chi-squared, corrected for ties, $= 6.04$ and 6.22, respectively). The mean ranks for the control, low- and high-lead treatments for the truncated and square root of the latencies were 17.2, 26.9, and 28.1, and 17.1, 27.3, and 27.9, respectively. The only significant difference was between the control versus the low- or high-lead treatments.

Significant interactions of lead by day ($Fs = 2.66, 2.90$, $df = 4/82$) for the latency measures were noted. As can be seen in Fig. 1 (for the square root of the latencies), a major source of the interactions was the increased latencies in the lead groups over days (especially Day 3 for the low-lead group) with respect to the control group. The only other significant ($p < .05$) interactions for the latency measures occurred between blocks and days ($Fs = 2.47, 2.80$, $df = 4/164$).

Four-way analyses of variance with two factors repeated indicated a significant interaction of block by day by lead treatment for both absolute and square root of the total errors per block ($Fs = 2.29, 2.10$, $df = 8/164$, $p \leq .05$). Marginally significant ($p = .05$) interactions of block by lead were also noted for both sets of data ($Fs = 2.10$, $df = 4/82$).

Four-way analyses of variance (lead, food, gender) with one factor repeated (days) for body weight over the three days of testing demonstrated significant ($p < .001$) gender ($F = 27.32$, $df = 1/35$) and day ($F = 6.14$, $df = 2/70$) differences. Analyses of body weight over the 2 wk. of the experiment indicated gender ($F = 32.53$, $df = 1/35$) and day ($F = 29.99$, $df = 2/70$) effects, with no major interactions. However, there were marginally significant ($p = .05$) differences in body weight between the two food conditions ($F = 5.70$, $df = 1/35$). The means and standard deviations of body weight for the six (lead and food) groups were: control-*ad lib.*: 31.5 $\pm$ 3.5 gm., control-restricted: 28.7 $\pm$ 2.8 gm.; low lead-*ad lib.*: 29.8 $\pm$ 3.3 gm.; low-lead-restricted: 27.4 $\pm$ 2.8 gm.; high-lead-*ad lib.*: 28.8 $\pm$ 2.5 gm.,

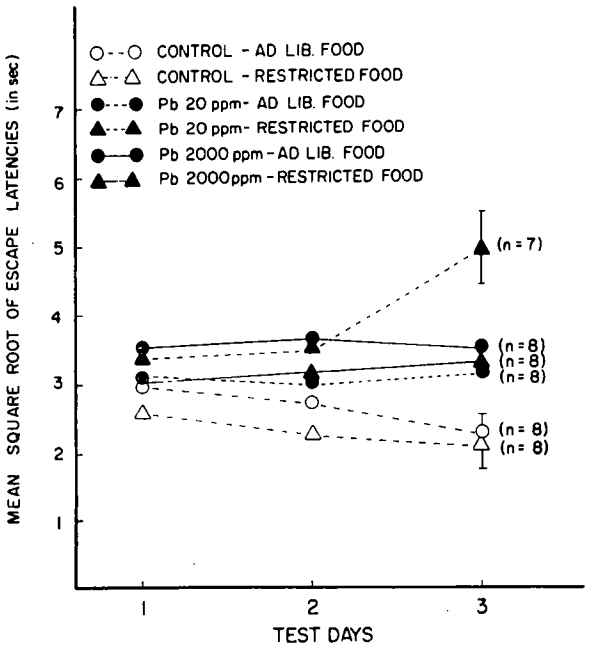
CONTROL - AD LIB. FOOD
CONTROL - RESTRICTED FOOD
Pb 20 ppm- AD LIB. FOOD
Pb 20 ppm- RESTRICTED FOOD
Pb 2000 ppm - AD LIB. FOOD
Pb 2000ppm - RESTRICTED FOOD
MEAN SQUARE ROOT OF ESCAPE LATENCIES (in sec.)
TEST DAYS
(n = 7)
(n = 8)
(n = 8)
(n = 8)
(n = 8)
(n = 8)

FIG. 1. Mean square roots of the escape latencies (in sec.) for the different lead treatments (tap water, 20 ppm Pb, ~2000 ppm Pb) and food conditions (*ad libitum* versus restricted) over the three test days in the experiment. Vertical bars indicate sample ± 1 standard errors of the mean (*SEM*). n = numbers of mice per group.

and high-lead-restricted: 27.8 ± 4.0 gm., respectively. None of the lead treatments significantly influenced body weight.

DISCUSSION

The present data indicate that experiments designed to evaluate dynamic processes statistically, i.e., repeated measures over time, can detect behavioral consequences of drinking low-level lead concentrations. Although the lead-treated mice displayed more errors and slower escape latencies relative to controls, the most significant behavioral effects from either 20-ppm or ~ 2000-ppm lead exposure were evident in the interactions of day by lead, block by lead, or day by block by lead. These effects were obtained *without* the confounding loss of body weight known to be associated with higher lead concentrations.

A first interpretation of the data is that lead treatments interfered with learning. If learning is defined in this instance as a progressive decrease in escape latencies over days of testing, then the 20-ppm and 2000-ppm groups did not learn compared to their controls. The control groups improved over the test days, but the lead groups remained the same or became worse.

There is another possibility. If we assume that latencies of the control mice were asymptotic by the first or second block of the first day (an assumption supported by the data), then no blatant learning curve would have been evident over days in the control groups. In fact, the *increased* swimming latencies over days displayed by the lead groups in both experiments suggests some other cumulative factor. Fatigue, attenuation of the aversive aspects of the task, or some indirect feature of swimming in the water are possibilities.

Enhanced variability within the scores of animals exposed to experimental treatments is a common observation (Persinger, Lafrenière, & Falter, 1976; Persinger & Fiss, 1978). In this study, both the 20-ppm and 2000-ppm lead groups demonstrated significantly greater variability in swim latencies than controls. Such variability was associated presumably with the differential sensitivity of individual animals to the lead. Sources of this variation were not grossly associated with body weight or with gender since neither factor was significantly correlated with or contributed to the swim latencies.

REFERENCES

BROWN, S., DRAGANN, N., & VOGEL, W. H. Effects of lead acetate on learning and memory in rats. *Archives of Environmental Health*, 1971, 22, 370-372.

BULLOCK, J. D., WEY, R. J., ZAIA, J. A., ZAREMBOK, I., & SCHROEDER, H. A. Effect of tetraethyl lead on learning and memory in the rat. *Archives of Environmental Health*, 1966, 13, 21-22.

OGILVIE, D. M. Sublethal effects of lead acetate on the Y-maze performance of albino mice (*Mus musculus L.*). *Canadian Journal of Zoology*, 1977, 55, 771-775.

PERSINGER, M. A., & FISS, T. B. Mesenteric mast cell degranulation is not essential for conditioned taste aversion. *Pharmacology, Biochemistry and Behavior*, 1978, 9, 725-730.

PERSINGER, M. A., LAFRENIÈRE, G. F., & FALTER, H. Oral taurine effects on inhibitory behavior: response transients to step-like schedule changes. *Psychopharmacology*, 1976, 49, 249-252.

QUARTERMAN, J. J., MORRISON, N., & HUMPHRIES, W. R. The effects of dietary lead content and food restriction on lead retention in rats. *Environmental Health*, 1976, 12, 180-187.

SCHMIDT, J. C., & CZECH, D. A. Effect of tetraethyl lead and restricted food intake on locomotor activity in the rat. *Pharmacology, Biochemistry and Behavior*, 1977, 7, 489-492.

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